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Life histories of guppies (*Poecilia reticulata* Peters, 1869; Poeciliidae) from the Pitch Lake in Trinidad

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Abstract.—Trinidadian guppies (*Poecilia reticulata*) are able to adapt to various environmental conditions and are even among the few species that can tolerate extensive pollution. In the Pitch Lake of Trinidad they live in highly toxic waters due to natural seepage of oil and bitumen. In this paper, we describe phenotypic divergence in several life-history traits between guppies from the Pitch Lake and from a nearby reference site with waters not polluted by bitumen/oil. We show that guppies from the Pitch Lake were (i) smaller and (ii) had a higher reproductive investment than those from the reference site. Furthermore, they (iii) produced more and smaller offspring. These results are congruent with a scenario of high mortality caused probably by a combination of water toxicity and higher predation than at the reference site. We therefore propose the Pitch Lake as an ideal system to study the effects of long-term (natural) water pollution on fishes, which might provide interesting insights into adaptation to extreme environments, and might further help to predict fish responses to anthropogenic pollution.

Keywords.— Life-history; Oil pollution; Predation; Phenotypic evolution.

Introduction

Among extremophile vertebrates (from the Latin term extremus and the Greek philia; roughly translating into "loving the extreme"), teleost fishes are particularly numerously represented and inhabit environments that are, for example, extreme due to salinities far above concentrations found in sea water, low oxygen content, low temperatures, the presence of toxins, or the absence of light (e.g., Gerday & Glansdorff 2009; Riesch et al. 2015b). While many of these habitats are naturally extreme to teleost fishes and can only be inhabited by highly specialized forms, more and more habitats, terrestrial and aquatic, are currently becoming extreme due to the impact of human activities on ecosystems in general and human-induced pollution in particular (e.g., Oziolor & Matson 2015; Reid et al. 2016; Hamilton et al. 2017).

The Pitch Lake (Fig. 1) in South-West Trinidad is the largest and best-known asphalt lake in the world (Trinidad and Tobago National Commission for UNESCO 2011; Schelkle et al. 2012). It was created and is maintained by natural upwelling of oils and bitumen that contain a plethora of hydrocarbons, sulphur compounds and metals, as well as volcanic ash, at concentrations high enough to create an extremely toxic environment (Richardson 1912; Ponnamperuna & Pering 1967; World Health Organization Concise International Chemical Assessment Document 2005). Due to the impermeability of the bitumen, several interconnected permanent pools fed by rainwater are interspersed throughout the Pitch Lake. Here, a surprising number of plants and animals seem to thrive, despite the fact that previous studies also reported relatively high water temperatures exceeding 30°C, coupled with high acidity and salinity (e.g., Mohammed et al. 2010; Schelkle et al. 2012). Nonetheless, the organisms inhabiting the Pitch Lake pools include bacteria (Meckenstock et al. 2014), invertebrates (Schelkle et al. 2012), amphibians

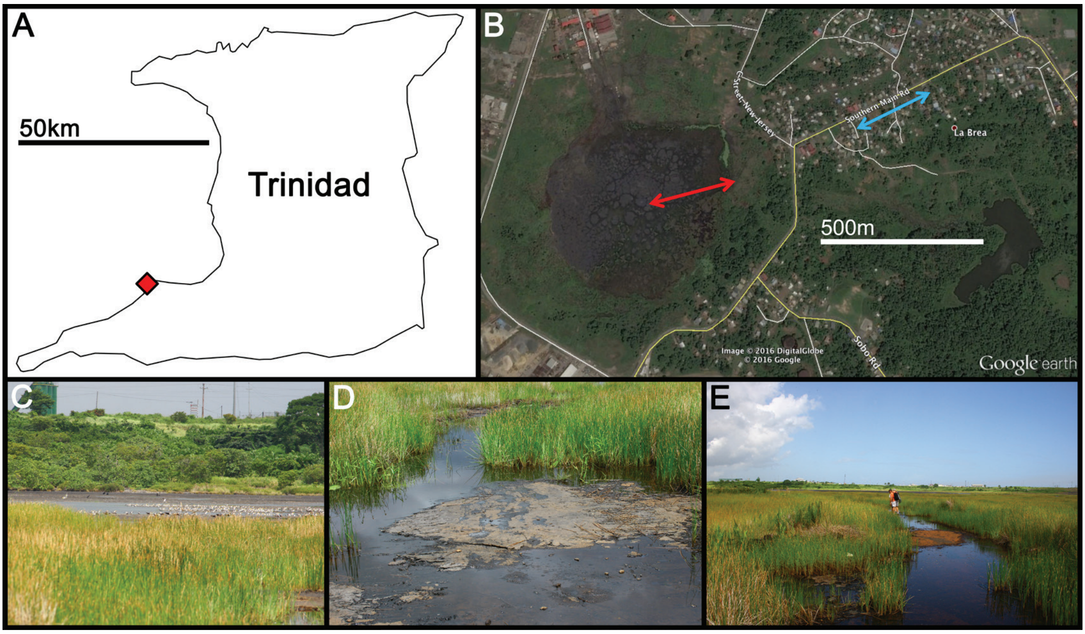

FIG. 1. (A) Location of our sample sites in Trinidad (red diamond); (B) Detailed view of collection transects. The red arrow indicates the general transect sampled within the Pitch Lake, while the blue arrow indicates the general transect sampled along a road-side ditch (our reference site); (C) large flocks of yellow-billed tern, *Sternula superciliaris*, and black skimmer, *Rynchops niger*, are resting and foraging on the Pitch Lake (i.e., the white 'dots' on the Pitch Lake); (D & E) Pitch Lake; (A) was created in R (R Core Team 2015), (B) was created using GOOGLE EARTH VS. 7.1.5.1557 (©2015 Google Inc., Mountain View, CA, USA), photos (C-E) by D. Bierbach.

(Schelkle et al. 2012) and fishes like Hart's killifish *Rivulus hartii* Boulenger, 1890, Guyana leaffish, *Polycentrus schomburgkii* Müller & Troschel, 1849, and the guppy, *Poecilia reticulata* Peters, 1869 (Mohammed et al. 2010; Schelkle et al. 2012).

The Trinidadian guppy has been a model organism for evolutionary and behavioural ecology for decades, largely due to the fact that as a result of human introductions, guppies now have an almost global distribution, but are also easy to keep and maintain in the laboratory (Evans et al. 2011; Magurran 2005). Furthermore, guppies are among those fish that can tolerate extensive natural and human-induced pollution and toxicity (Araujo et al. 2009; Riesch et al. 2015b). The guppies at the Pitch Lake in particular have recently been used by Schelkle and colleagues (2012) to study the influence of the pitch-induced toxicity on parasite dynamics. The study showed that the Pitch Lake water effectively protects guppies from microbial and gyrodactylid parasite infections (see also the enemy release hypothesis; Williamson 1996). Also, since fishes seem to naturally produce enzymes that, to a certain extent, allow them to detoxify different hydrocarbons when exposed during pollution events (Lee et al. 1972; Neff et al. 1976; Ownby et al. 2002), there should be a high potential for fishes to colonize extreme, oil-polluted environments and even locally adapt to oil contamination. Such local adaptations of fishes to polluted waters has been described for several other species and localities (Reid et al. 2016; Hamilton et al. 2017).

In the current study, we analyze phenotypic divergence in life-history traits in Pitch Lake guppies by comparing them to a nearby reference population living in a roadside ditch outside of the oil- and bitumen-contaminated area of the Pitch Lake (Fig. 1). A recent study by Rolshausen and colleagues (2015) revealed that oil-polluted waters negatively impact guppy survival. Moreover, we would further expect detoxification of hydrocarbons and other toxins associated with oil pollution to be energetically costly (e.g., Marchand et al. 2004; Passow et al. 2015). As a result, we expect guppies from the Pitch Lake to suffer increased mortality across age classes when compared to those from the reference population, and therefore express an *r*-selected phenotype, which should be associated with a decreased investment into growth and an increased investment into reproduction (Pianka 1970). In other words, we predict guppies from the Pitch Lake to be smaller (reduced standard length and size-specific lean weight) and to have less body fat than their counterparts from our non-polluted reference site, but to have a higher gonadosomatic index (GSI), and reproductive allocation (RA), and to produce more and smaller offspring (Reznick et al. 2002a). Alternatively, if larger offspring size were to convey a fitness advantage in oil polluted sites (as has been suggested for other environmental toxins such as hydrogen sulphide; Riesch et al. 2016) then Pitch Lake guppies should be characterized by larger offspring size coupled with a lower fecundity compared to guppies from non-polluted sites.

Materials and Methods

We collected fish in June 2012 using small dip nets along two sampling transects in the Pitch Lake and the nearby reference site (Figure 1). Collections were made under a permit to M.S. kindly supplied by the Directorate of Fisheries of the Ministry of Food Production, Land and Marine Affairs of the Republic of Trinidad and Tobago, issued May 31st, 2012. Caught fish (for sample sizes and some habitat parameters, please refer to Table 1) were immediately euthanized with an overdose of clove oil and preserved in 10% formalin solution. Similar to previous studies (e.g., Mohammed et al. 2010; Schelkle et al. 2012), we also found the Pitch Lake to be highly acidic compared to our reference site, while water temperatures tended to be slightly higher in the Pitch Lake as well; although it was high (>30ºC) also at our reference site (Table 1). However, due to logistic constraints, we were not able to directly test for toxicity levels in each habitat. We therefore cannot completely rule out that even the reference site, due to its proximity to a main road, might suffer from some toxicity as a result of oil- and gasoline-runoff from the road.

Dissections to collect male, female, and offspring-related life-history traits followed well-established protocols (Reznick & Endler 1982; Riesch et al. 2013, 2016). We collected the following male and female life-history traits: standard length (SL [mm]), dry weight [mg], lean weight [mg], and fat content [%]. For males we also calculated the gonadosomatic index, GSI [%] (i.e., testis dry weight divided by the sum of reproductive tissue dry weight and somatic dry weight), while for females we quantified fecundity (number of developing offspring), offspring dry weight [mg], offspring lean weight [mg], offspring fat content [%], and reproductive allocation, RA [%] (i.e., offspring dry weight divided by the sum of offspring dry weight plus somatic dry weight).

Prior to statistical analyses, we log10-transformed (male and female SL, male and female lean weight, and embryo dry and lean weight), square root-transformed (fecundity), or arcsine(square root)-transformed (male and female fat content, male and female GSI, embryo fat content) all life-history variables, and conducted subsequent z-transformation to meet assumptions of statistical analyses (i.e., these transformations facilitated normality of model residuals). Z-transformed variables were used in all subsequent analyses unless specified otherwise.

We first tested for differences in body size (SL) by running an ANOVA with sex (male vs. female) and population (the Pitch Lake vs. reference site) as factors. We then performed sex-specific multivariate general linear models (GLMs) on all other life-history traits (sex-specific lean weight and fat content, female RA and fecundity, male GSI, embryo lean weight and embryo fat content) with population as the fixed factor of interest. In the GLM on male life-history traits, only SL was included as covariate, while SL and embryonic stage of development

Table 1. Mean±SE of male and female life-history traits in wild-caught guppies from the Pitch Lake (pH: 6.06; water temperature: 29.2-33.9ºC; conductivity: 109µS) and a reference site (pH: 9.35; water temperature: 30.7ºC; conductivity: 499µS) in Trinidad. M: males; F: females; GSI: gonadosomatic index; RA: reproductive allocation; MI: matrotrophy index.

Population	Coordinates	Sex	N^a	SL [mm]	Lean weight [mg][b]	Fat content [%]	Fecundity[b]	Estimated embryo dry weight at birth [mg][c]	Embryo fat content [%]	GSI [%]	RA[b] [%]	MI
Pitch Lake	N 10°14.084'	M	8 / 8	14.96±0.17	19.90±0.68	2.52±1.26	-	-	-	2.74±0.20	-	-
	W 61° 37.469'	F	11 / 18	20.84±0.57	74.12±2.80	4.24±1.07	16.70±2.54	0.49	20.62±2.40	-	17.86±1.91	0.53
Reference site	N 10° 14.262'	M	21 / 21	16.48±0.22	21.66±0.38	6.56±0.80	-	-	-	1.96±0.13	-	-
	W 61° 37.190'	F	24 / 25	24.93±0.75	76.49±1.79	12.80±3.46	10.60±1.62	0.92	19.97±0.78	-	12.00±1.28	0.78

[a] the numerator corresponds to reproductively active males and females & the denominator equals the total number of collected and dissected males and females.
[b] lean weight and fecundity are given as estimated marginal means corrected for differences in SL (evaluated at SL = 16.06 mm for males and SL = 23.65 mm for females), while RA-values are estimated marginal means corrected for differences in embryonic stage of development (evaluated at stage 25; Riesch et al. 2011).
[c] estimated embryo dry weight at birth is calculated using the slope and intercept from the regression between log-transformed embryonic dry mass and stage of development.

served as covariates in the model on female life-history traits.

To evaluate the mode of maternal provisioning, we calculated the matrotrophy index (MI) using the slopes and intercepts from the regression analysis described below. The MI equals the estimated dry mass of the embryo at birth divided by the estimated dry mass of the ovum at fertilization (e.g., Reznick et al. 2002b; Riesch et al. 2011, 2013). If the eggs were fully provisioned by yolk before fertilization (lecithotrophy), then we would expect the embryos to lose 25%–40% of their dry mass during development (MI between 0.60 and 0.75; Scrimshaw 1945; Wourms 1981). On the other hand, in the case of continuous maternal provisioning after fertilization (matrotrophy), one would expect the embryos to lose less weight (MI between 0.75 and 1.00) or to even gain weight during development (MI > 1.00; e.g., Reznick et al. 2002b). Thus, maternal provisioning was evaluated by analyzing the relationship between log-transformed embryonic dry mass and stage of development by means of linear regression analysis (Reznick et al. 2002b; Riesch et al. 2011, 2013).

Results

In the ANOVA on body size, we found that sexes ($F_{1,60}$= 109.702, P< 0.001) and populations differed from one another ($F_{1,60}$= 16.815, P< 0.001), while the interaction sex-by-population was not significant ($F_{1,60}$= 3.565, P= 0.064). Male guppies had a smaller mean body size than females, and Pitch Lake guppies were smaller than guppies from the reference population (Fig. 2A & B).

In the multivariate GLM on male life-history traits we found a significant effect of both the covariate SL ($F_{3,24}$= 52.523, P< 0.001, Partial η^2= 0.868) and the factor population ($F_{3,24}$= 8.610, P< 0.001, Partial η^2= 0.518). Trait-specific *post-hoc* ANOVAs revealed that the effect of SL was due to a size-effect on lean weight ($F_{1,26}$= 166.490, P< 0.001), while fat content ($F_{1,26}$= 0.024, P= 0.879) and GSI ($F_{1,26}$= 0.518, P=0.478) were not affected; however, all traits were significantly different between both populations (lean weight: $F_{1,26}$= 11.869, P= 0.002; fat content: $F_{1,26}$= 7.728, P= 0.010; GSI: $F_{1,26}$= 7.927, P=0.009; Fig. 2A).

In the multivariate GLM on female life-history traits we found significant effects of the covariates SL ($F_{6,26}$= 235.297, P< 0.001, Partial η^2= 0.982) and embryonic stage of development ($F_{6,26}$= 5.899, P= 0.001, Partial η^2= 0.576), as well as the factor population ($F_{6,26}$= 5.817, P= 0.001, Partial η^2= 0.573). *Post-hoc* ANOVAs revealed that SL had a significant effect on lean weight ($F_{1,31}$= 1587.961, P< 0.001), fecundity ($F_{1,31}$= 18.318, P< 0.001) and embryo lean weight ($F_{1,31}$= 15.140, P< 0.001); whereas fat content ($F_{1,31}$= 0.167, P= 0.686), embryo fat content ($F_{1,31}$=0.678, P= 0.416) and RA ($F_{1,31}$=0.486, P=0.491) were not affected (Fig. 2B). Moreover, stage had a significant effect on fecundity ($F_{1,31}$= 13.784, P= 0.001), embryo lean weight ($F_{1,31}$= 10.301, P= 0.003) and RA ($F_{1,31}$=25.092, P< 0.001), while it did not affect the other traits (lean weight: $F_{1,31}$= 0.000, P= 0.986; fat content: $F_{1,31}$=0.189, P=0.667; embryo fat content: $F_{1,31}$=0.112, P= 0.740; Fig. 2C). On the population level, we found significant differences in lean weight ($F_{1,31}$= 29.296, P< 0.001), embryo lean weight ($F_{1,31}$= 12.185, P= 0.001) and RA ($F_{1,31}$= 4.352, P= 0.045); however, there were no significant differences in fat content ($F_{1,31}$=3.274, P=0.080), embryo fat content ($F_{1,31}$=0.361, P= 0.552) or fecundity ($F_{1,31}$= 3.804, P= 0.060; Fig. 2B).

According to the MI, both populations were

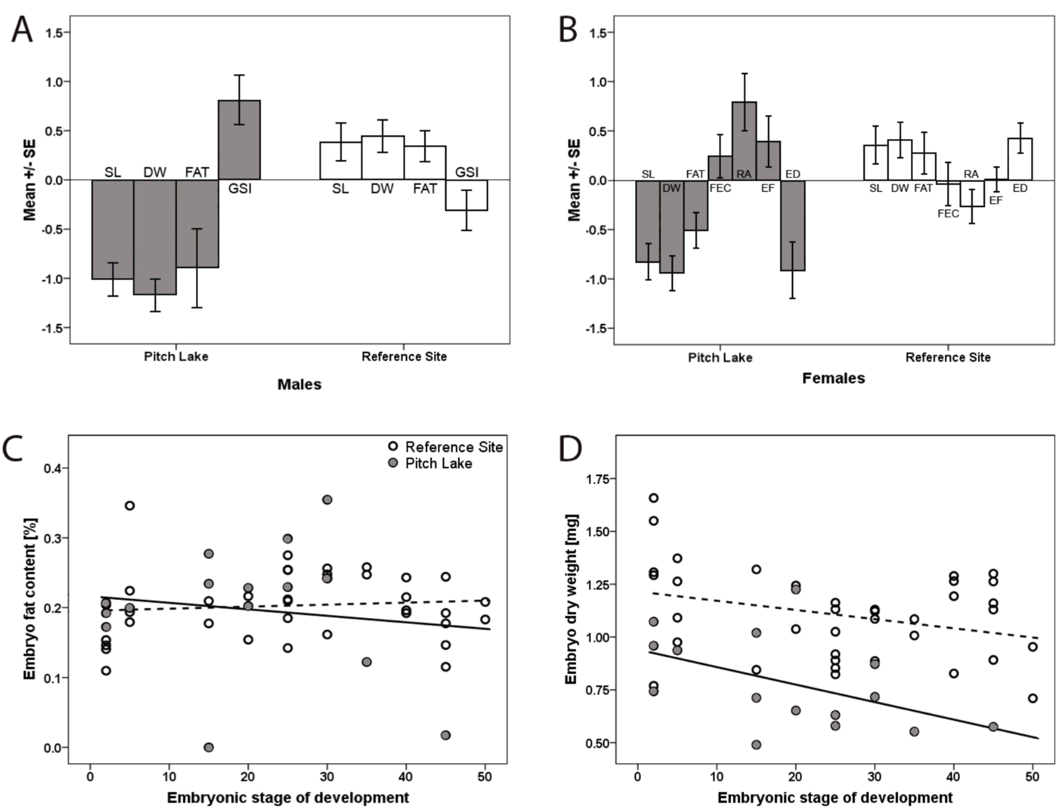

FIG. 2. Mean z-transformed life-history traits of male (A) and female (B) guppies. SL: standard length, DW: dry weight, FAT: fat content, GSI: gonadosomatic index, FEC: fecundity, RA: reproductive allocation, EF: embryo fat content, and ED: embryo dry weight. Scatter plots depicting (C) embryo fat content and (D) embryo dry weight during the course of embryo development.

characterized by a lecithotrophic provisioning strategy, and guppies from the reference site produced offspring nearly twice as heavy at birth as Pitch Lake guppies (Table 1; Fig. 2D).

Discussion

Overall, life histories quantified for both guppy populations were within the range of those previously reported for Trinidadian guppies, although some trait values in Pitch Lake guppies, such as estimated offspring size at birth and MI, were on the lower end of those previously reported (e.g., Reznick & Bryga 1987; Reznick et al. 1996; Pires et al. 2010).

We largely found our a priori predictions confirmed: guppies from the Pitch Lake were smaller (SL), had lower lean weight and fat content, and higher GSI (males) and RA (females) compared to guppies from the reference population. Furthermore, females from the Pitch Lake produced more and smaller offspring than the reference-site females. This is congruent with a scenario of higher mortality across age classes in the Pitch Lake, similar to what has been reported in several river systems in southern Trinidad by Rolshausen and colleagues (2015) who investigated guppy responses to human-induced crude-oil PAH (polycyclic aromatic hydrocarbon) pollution.

In agreement with previous studies, our environmental data indicated that the Pitch Lake was highly acidic, and in at least some places had higher water temperatures compared to our reference site. Previous work on livebearing fishes suggested that lower pH should result in increased fecundity, lean weight and body size (e.g., Riesch et al. 2015a), while higher water temperatures should also result in higher fecundity and greater investment into reproduction (e.g., Vondracek et al. 1988; McManus & Travis 1998). Our results are in partial agreement with this, as the low pH and slightly higher water temperatures of the Pitch Lake were associated with higher fecundity and greater investment into reproduction, but patterns of body size and lean weight were opposite to those associated with pH in a previous study on several species of *Gambusia* (Riesch et al. 2015a). However, we currently do not know if these differences in pH and temperature between the sites are temporally stable or unique to our specific time of sampling.

Interestingly, we did not find any evidence for selection for larger offspring size coupled with a reduced fecundity, as is often the case in hydrogen sulphide (H_2S) toxic waters, even though Richardson (1912) reported the presence of traces of H_2S also in the Pitch Lake. One possible explanation for this is that the concentration of H_2S is too low to elicit increased offspring size (for a similar scenario see for instance Riesch et al. 2016). Alternatively, other toxins in the water or other selective agents might largely select for increased fecundity coupled with smaller offspring size, and this simply overrides H_2S-induced selection.

One such environmental factor that might differ between the Pitch Lake and our reference site, and might camouflage phenotypic responses to toxin-induced selection, is predation. Personal observations during sampling and previous studies (Mohammed et al. 2010; Schelkle et al. 2012) suggest that the Pitch Lake is likely to be a high predation environment due to a high abundance of piscivorous birds (among them kingfishers, herons, egrets, yellow-billed tern, *Sternula superciliaris*, and black skimmer, *Rynchops niger*; Fig. 1C), the presence of several cichlids, Guyana leaffish, as well as a relatively high density of piscivorous *Rivulus hartii*. In contrast, guppies were the only fish caught and observed at the reference site (D. Bierbach and M. Schartl, personal observation). In high-predation sites, guppies are usually characterized by high levels of extrinsic mortality, which also translates into *r*-selected phenotypes. In fact, Pitch Lake guppies exhibited all life-history traits typical for poeciliids in high-predation environments: small offspring size coupled with high fecundity, high investment into reproduction, smaller body size, and reduced body fat (*P. reticulata*: Reznick & Endler 1982; *Brachyraphis rabdophora*, Regan, 1908: Johnson & Belk 2001; *Gambusia hubbsi*, Poey, 1854: Riesch et al. 2013). Therefore, while our results are largely in line with our a priori predictions regarding bitumen-based toxicity (see above), they are also congruent with this interpretation of selection due to predation. Future studies should attempt to more clearly separate the different effects that predation, pH, water temperature, productivity, and food availability, might have on guppies from Pitch Lake and other sites.

Oil pollution, usually caused by human activities, is an extremely detrimental environmental factor for aquatic habitats (Neff 1987). Studying the effect of oil pollution on population dynamics of aquatic organisms has therefore important consequences for the conservation of aquatic environments (Hamilton et al. 2017). Trinidad in particular has a long history of oil exploitation that causes human-induced oil pollution in many rivers (Rolshausen et al. 2015). In this regard, guppies in the Pitch Lake present a unique study opportunity, since they inhabit a very peculiar place that experiences natural oil pollution. Future studies should test if Pitch Lake guppies might even be locally adapted to this harsh environment.

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